Association Between Novel Circulating Biomarkers of Cytochromes P450-derived Eicosanoid Metabolism and Prognosis in Patients with Established Atherosclerotic Cardiovascular Disease

Melody Robbins, PharmD Candidate,[1]

in collaboration with

Akinyemi Oni-Orisan, PharmD, PhD,[1] Rebecca Bacheler, MS,[1] Alexis Williams, PharmD,[1]
Matthew L. Edin, PhD,[2] George A. Stouffer, MD,[3] Darryl C. Zeldin, MD,[2]
and Craig R. Lee, PharmD, PhD[1]

[1]Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC.
[2]National Institute of Environmental Health Sciences
[3]Division of Cardiology; School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Abstract

Introduction: Atherosclerotic cardiovascular disease (ASCVD) is a major health problem that requires new therapeutic strategies. Cytochrome P450 (CYP) enzymes metabolize arachidonic acid to form epoxyeicosatrienoic acids (EETs), which exhibit cardioprotective effects in preclinical models. CYP-derived EETs are rapidly hydrolyzed by soluble epoxide hydrolase (sEH) into less active dihydroxyeicosatrienoic acids (DHETs). Inhibition of sEH has been proposed as a novel ASCVD treatment. However, the relationship between EET concentrations and ASCVD prognosis in humans has not been fully investigated. The purpose of this study was to evaluate the relationship between EETs and risk of future major adverse cardiovascular events (MACE).

Methods: A secondary analysis was conducted on an existing cohort of 162 participants that underwent coronary angiography from 2012 to 2014. Plasma EET and DHET metabolite concentrations were quantified by liquid chromatography tandem mass spectrometry. The occurrence of MACE (defined as death, acute coronary syndromes [ACS], ischemic stroke or a revascularization procedure) was retrospectively collected from the electronic health record. The time to MACE or last follow-up from the initial catheterization lab visit was calculated. In participants with available follow-up data and angiographic evidence of ASCVD at baseline (N equals 98), the relationship between baseline plasma EET concentrations (as well as sum EETs plus DHETs [a biomarker of CYP function] and 14,15-EET to DHET ratio [a biomarker of sEH function]), and risk of future MACE was evaluated using proportional hazards regression. These relationships were examined across low, medium, and high tertiles for each biomarker of interest. The analyses were adjusted for covariates that were associated with EET concentrations and/or ASCVD outcomes. Data are presented as percentages, median [interquartile range], or adjusted hazard ratio (HR) [95 percent confidence interval (CI)]. A p-value of less than 0.05 was considered statistically significant.

Results: The ASCVD study population, on average, was 65 years old, 42 percent female, 16 percent African-American, and 49 percent had a prior revascularization procedure. During a median follow-up of 3.8 years, 37 participants (38 percent) experienced a MACE. No significant difference was observed in baseline plasma EET concentrations across those with (0.61 ng/mL [0.52 to 0.71]) versus without (0.66 ng/mL [0.54 to 0.83]) a future MACE (p equals 0.406). Similarly, there were no significant differences in either baseline sum EETs plus DHETs (p equals 0.363) or 14,15-EET to DHET ratios (p equals 0.641). Compared to participants in the high EET concentration tertile group, those in the combined medium and low tertile group appeared to have a higher risk of future MACE (27 percent [9 of 33] versus 43 percent [28 of

65], respectively; adjusted HR 1.64 [0.78 to 3.80], p equals 0.201). However, the observed trend was not statistically significant. A similar association with future MACE risk was observed with sum EETs plus DHETs (24 percent versus 45 percent; adjusted HR 1.98 [0.90 to 4.87], p equals 0.094), but not 14,15-EET to DHET ratios (38 percent versus 36 percent; adjusted HR 0.94 [0.44 to 1.91], p equals 0.866).

Conclusions: No statistically significant associations were found between baseline plasma EET metabolite concentrations and the risk of future MACE. Compared to participants in the high tertile group, those in the low or medium EET and sum EETs plus DHETs tertiles may have a higher risk of future MACE; however, these findings were not statistically significant and should therefore be interpreted with caution. If a relationship between EET concentrations and occurrence of future MACE exists, the data suggests this may be attributed to lower CYP function rather than higher sEH function. Additional investigation in a larger population is warranted.

Introduction

Atherosclerotic cardiovascular disease (ASCVD) is a major public health problem in the United States. An estimated 16.5 million Americans ≥ 20 years old have ASCVD[1]. Cardiovascular disease including ASCVD is one of the leading causes of death globally (31% of all deaths) and in the United States[1]. Major adverse cardiovascular events (MACE) associated with ASCVD include death, acute coronary syndrome (ACS), ischemic stroke, and progressive disease requiring a coronary artery revascularization procedure (revasc) such as percutaneous coronary intervention (PCI) with stenting or coronary artery bypass graft (CABG) surgery. Despite current therapies, patients with ASCVD are still at high risk of experiencing MACE and having increased morbidity and mortality resulting from those events. Thus, new treatment strategies are needed to improve the prognosis in ASCVD patients by decreasing the incidence of MACE and reducing the morbidity and mortality of ASCVD.

Precision medicine consists of treatment strategies that are based off of the individual variability of patients[2]. This approach to therapy has already been successful in targeting novel therapies to distinct oncogenic mechanisms[2] for cancer treatment, but it has not been as largely utilized in the cardiovascular field. People at high risk of a detrimental cardiovascular outcome with dysfunctions in bioactive pathways could be identified; then biomarkers of these pathways could be used to determine which population subsets are at high risk of a detrimental outcome, which may be lessened by a therapy that targets the bioactive pathway. Candidate pathways are needed for a precision therapy strategy in ASCVD[3]. Research needs to be done to discover populations that will benefit from the development of precision medicine strategies in order to improve prognosis of ASCVD.

It is well established that cyclooxygenase (COX)- and lipoxygenase (LOX)- mediated metabolism of arachidonic acid to bioactive prostaglandin and leukotriene metabolites, respectively, is important in the regulation of cardiovascular function[4,5]. Compared to these well-known pathways, arachidonic acid metabolism by cytochrome P450 (CYP) enzymes into bioactive epoxyeicosatrienoic acids (EETs) has emerged as a novel regulator of cardiovascular function and potential therapeutic target for ASCVD. CYP-derived EETs have been shown to have potent cardiovascular protective effects including vasodilation and inhibition of myocardial and vascular inflammatory responses in preclinical models[4-10]. However, EETs are rapidly metabolized by soluble epoxide hydrolase (sEH) into less active dihydroxyeicosatrienoic acid (DHET) metabolites[4]. Inhibition of sEH increases cellular and circulating EET concentrations, promotes the biological effects of EETs, and has demonstrated potent cardiovascular protective effects in preclinical models of hypertension, vascular injury, atherosclerosis, and myocardial

ischemia-reperfusion injury[11-14]. Based on these models, sEH inhibition has been proposed[15] as a potential cardiovascular protective strategy in humans that could potentially benefit from a precision medicine approach.

Despite the ongoing preclinical and clinical development of sEH inhibitors for non-ASCVD indications[16,17], there is limited knowledge of the relevance of the CYP epoxygenase pathway of arachidonic acid metabolism in human ASCVD. Our research team and others have previously demonstrated that genetic polymorphisms in CYP epoxygenases and sEH are associated with the development of ASCVD, and low circulating EET levels are associated with the presence and extent of ASCVD in patients undergoing coronary angiography[18-22]. However, the relationship between EET levels and occurrence of future cardiovascular events in ASCVD patients has never been evaluated.

Therefore, the objectives of this study were to define the incidence of MACE in an established cohort of ASCVD patients with existing eicosanoid metabolite data[22], and evaluate the relationship between EET metabolite concentrations at baseline and risk of future MACE.

Methods

Study population

A cohort of 162 participants 18-80 years of age that underwent a coronary angiography procedure at the University of North Carolina (UNC) cardiac catheterization laboratory were identified as described[22] from September 2012 to February 2014. The exclusion criteria for this previously established cohort were severe concurrent illness, systemic inflammatory disease, cancer actively being treated, hematologic disorders affecting platelet function, prior heart transplantation, hematocrit <30%, ST segment elevation myocardial infarction (STEMI), end-stage liver disease, end-stage renal disease on dialysis, and systemic immunosuppressive medication use (i.e. corticosteroid use).

Whole blood was drawn from an indwelling arterial catheter during the coronary angiography procedure, but before initiation of a PCI procedure (if indicated). The plasma was separated immediately by centrifugation and then stored at a temperature of -80°C. The study protocol was approved by the UNC Biomedical Institutional Review Board and eligible participants provided written informed consent.

Quantification of eicosanoid metabolite concentrations

Arterial plasma samples were previously analyzed for eicosanoid metabolites by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described[22]. Data were generated for 28 metabolites of interest, including arachidonic acid (AA)-derived CYP epoxides (EETs) and AA-derived sEH diols (DHETs). The AA-CYP epoxide metabolites of particular interest are regioisomers 14,15-EET, 11,12-EET, and 8,9-EET (the 5,6-EET regioisomer is unstable and not quantifiable[22]). The AA-sEH diols of interest are regioisomers 14,15-DHET, 11,12-DHET, 8,9-DHET, and 5,6-DHET. Additional metabolites, including AA-derived CYP hydroxyls, AA-derived LOX metabolites, AA-derived COX metabolites, linoleic acid (LA)- derived LOX metabolites, LA-derived CYP epoxides, LA-derived sEH diols, and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA)- derived epoxide/diols, are also quantified on the panel.

Three CYP epoxygenase pathway biomarkers were calculated for each participant: sum EETs, sum EETs + DHETs, and 14,15-EET:DHET ratio, as previously described[21,22,27]. In order to calculate the sum total concentration of EETs, the 14,15-EET, 11,12-EET, and 8,9-EET metabolites, which exhibit high inter-metabolite correlations, were summed for each participant to calculate the total EET concentration (sum EETs). This variable is reflective of the total circulating EET concentration in each individual participant. The sum EETs + DHETs were calculated by adding the 14,15-EET, 11,12-EET, 8,9-EET, 14,15-DHET, 11,12-DHET, 8,9-

DHET, and 5,6-DHET metabolites in each participant. This represents CYP epoxygenase function because CYP epoxygenases convert arachidonic acid into EET metabolites which are then converted into DHETs by sEH[4,5] (**Supplemental Figure 1**). In order to calculate the 14,15-EET:DHET ratio, the metabolite 14,15-EET was divided by the metabolite 14,15-DHET for each participant. This ratio is a biomarker of sEH activity because EET metabolites are converted to DHETs by sEH[4] and 14,15-EET is the preferred substrate for sEH[5] (**Supplemental Figure 1**).

Collection of clinical outcome data from the electronic health record

The electronic health record (EPIC) was retrospectively evaluated to identify hospitalizations for up to 4 years of follow-up after the participant's initial visit for the coronary angiography procedure (baseline). Twenty-one participants did not have a follow-up visit in the electronic health record, were considered lost to follow-up, and were excluded from the outcome analysis.

MACE + revasc was defined as a composite outcome of death from any cause, hospitalization due to non-fatal acute coronary syndrome [ACS] (unstable angina [UA], non-ST elevation myocardial infarction [NSTEMI], or ST-elevated myocardial infarction [STEMI]), hospitalization due to a non-fatal cerebrovascular event (ischemic stroke or transient ischemic attack [TIA]), or hospitalization due to a coronary artery revascularization procedure including percutaneous coronary intervention [PCI] or coronary artery bypass grafting [CABG]. All potential events were verified by at least 2 individuals. The date of each event was recorded as well as the date of the last follow-up visit as shown in the electronic health record. In individuals without an event, follow-up was censored at the last available encounter in the medical record during 4 years of follow-up.

Statistical analysis

Data are presented as a mean ± standard deviation, median (interquartile range [IQR]), or count (%) unless otherwise indicated. Prior to analysis, metabolite concentrations and EET: DHET ratios were log transformed because the data were not normally distributed. Statistical analyses were performed using JMP Pro 12. A p-value < 0.05 was considered to be statistically significant.

The number and frequency of participants that experienced a MACE + revasc event over the 4-year follow-up period was calculated in the cohort of 141 participants with follow-up data in the electronic health record. The median (interquartile range) for the time to MACE + revasc or last follow-up from the initial catheterization lab visit, and the number and frequency of each

individual component of the MACE + revasc composite were also calculated for each participant.

Demographic and clinical factors were collected from the participants as previously described[22]. This included variables such as demographic factors (gender, race, age), comorbidities and risk factors for ASCVD (cigarette smoking status, diabetes, hypertension, hyperlipidemia, prior cardiovascular events, obese BMI), and indicators of existing ASCVD (maximum coronary artery stenosis, prior revascularization procedure). The number and frequency of each clinical and demographic factor was reported for the cohort of 141 participants. Then, those participants were further stratified into those with (N=98) and without (N=43) significant ASCVD at baseline (defined as having current ≥ 50% stenosis or a prior revascularization procedure in a major coronary artery). The number and frequency of each demographic and clinical variable was also reported within the significant ASCVD strata.

Study population characteristics were compared across those with and without a MACE + revasc event using a student's t-test for continuous data and chi-squared or Fisher's exact test for categorical data as appropriate. Since 37 of the 41 MACE + revasc events that were identified occurred in the strata of patients with significant ASCVD at baseline (N=98), we performed all further described analyses in this population.

Sum EETs, sum EETs + DHETs, and 14,15-EET:DHET ratios (log-transformed) were compared across those with and without a future MACE + revasc event using a student's t-test. Sum EETs was the primary eicosanoid metabolite endpoint, while the sum EETs + DHETs and the 14,15-EET:DHET ratio were secondary endpoints. Each biomarker was then divided into tertiles, which were classified as low, medium, and high. Participant characteristics were compared across the tertiles to determine if confounding variables existed between the groups using an analysis of variance for continuous data and chi-squared or Fisher's exact test for categorical data as appropriate. The relationship between tertiles and time to occurrence of a future MACE + revasc event was assessed using Cox proportional hazards regression. The hazard ratio (HR) and 95% confidence intervals (CIs), relative to the low tertile, were calculated for each biomarker. As a secondary analysis, the low and medium (referred to as low/med) tertiles were combined and the relationship between that new group and the high tertile group was determined using Cox proportional hazards regression. Kaplan-Meier curves were generated using GraphPad Prism 7.

The relationship between demographic and clinical factors and the occurrence of future MACE + revasc events was assessed using Cox proportional hazards regression. The variables identified that were associated with EET concentrations and/or ASCVD outcomes were used to

create a multivariable adjusted model for the analyses described above. The model adjusted for demographic characteristics (age, race, sex) and confounding variables (history of diabetes, history of hypertension, prior revascularization procedure, current obstructive ASCVD, history of smoking, and obesity) that were associated with EET concentrations and/or ASCVD outcomes.

Results

Study population

The baseline characteristics of participants in this study are shown in **Table 1**. In the participants with follow-up (N=141), 98 (70%) exhibited significant ASCVD at the time of the baseline cardiac catheterization. The age median for the ASCVD population was 65 (53-71) years old with 42% being male and 16% being African American. Some of the risk factors for ASCVD that participants had were hypertension (80%), hyperlipidemia (68%), and prior revascularization (49%) indicating progressive disease. A comparison of clinical and demographic factors across participants with and without a future MACE + revasc event is shown in **Supplemental Table 1**. Notably, participants with a prior revascularization procedures were significantly more likely to experience a future MACE + revasc event (p=0.014).

Incidence of MACE + revasc in the study population

In **Table 2**, the incidence of events over the 4-year follow-up period is shown in the entire study population with follow-up data (N=141) and the strata of participants with significant ASCVD at baseline (N=98). Out of the 41 events that occurred in the follow-up population (N=141), 37 (90%) of those occurred within the ASCVD population (2 deaths, 11 non-fatal ACS events, 3 non-fatal stroke/TIA events, and 21 revascularization procedures). Overall, the percentage of participants who had a MACE + revasc event in the ASCVD population was 37.8%. The majority of events that occurred were revascularization procedures. The median (IQR) time to MACE + revasc or last follow-up in the ASCVD population was 2.5 (0.8-3.9) years.

Comparison of clinical factors across eicosanoid metabolite tertiles

Comparisons of clinical factors across sum EETs tertiles were completed to determine whether the data was affected by any confounding variables (**Table 3**). The age of the participants (p=0.025), obesity status (p=0.039), smoking status (p=0.039), and ACS upon presentation to the catheterization lab (p=0.028) differed significantly between the tertiles. Participants in the low tertile tended to be younger and more obese than the participants in the medium and high tertiles. More participants had a recent smoking history in the medium tertile compared to the low and high tertiles. Participants who presented to the lab with ACS events were mostly in the low and high groups as compared to the medium tertile group, respectively. Secondary comparisons across tertiles were performed in the sum EETs + DHETs tertiles (**Supplemental Table 2**) and 14,15-EET:DHET ratio tertiles (**Supplemental Table 3**). No

clinical factors were significantly different across sum EETs + DHETs, but age was significantly different across the 14,15-EET:DHET ratio tertiles.

Comparison of eicosanoid metabolites across future MACE + revasc event status

Within the ASCVD population, no significant differences in sum EETs, sum EETs + DHETs, or 14,15-EET:DHET ratio concentrations at baseline were observed between participants that experienced a future MACE + revasc event versus those that did not (**Table 4**).

Sum EET concentration tertiles at baseline were not significantly associated with incidence of a MACE + revasc event (log rank p=0.279) (**Figure 1**). As compared to the high sum EETs tertile event rate (27%), the risk of experiencing a MACE + revasc event in the low sum EETs tertile group (39%) (adjusted HR 1.32, 95% CI 0.53-3.39, p=0.553) and the medium sum EETs tertile group (47%) (adjusted HR 2.03, 95% CI 0.85-5.10, p=0.110) was not statistically significant. The low/med group event rate of 43% as compared to the high sum EETs tertile event rate (adjusted HR 1.64, 95% CI 0.78-3.80, p=0.201) was not statistically significant as well.

There appeared to be a trend toward a higher incidence of future MACE + revasc events in the medium and low sum EETs + DHETs tertiles compared to the high tertile (log rank p=0.313) (**Figure 2A**). Compared to the high sum EETs + DHETs tertile event rate (24%), the participants in the low tertile (36%) (adjusted HR 1.51, 95% CI 0.58-4.08, p=0.400) and the medium tertile (53%) (adjusted HR 2.58, 95% CI 1.05-6.82, p=0.038) appeared to be at higher risk of a MACE + revasc event, although the low tertile comparison was not significant. The participants in the low/med sum EETs + DHETs group also appeared to have a higher event rate (45%) than the high tertile group (adjusted HR 1.98, 95% CI 0.90-4.87, p=0.094). There was no association between 14,15-EET:DHET ratio tertiles and incidence of future MACE + revasc events (log rank p=0.593) (**Figure 2B**).

Discussion

CYP-derived EETs have been shown to have potent protective effects in preclinical models[4-10] and low circulating concentrations have been associated with the presence of significant ASCVD[22]. However, the relationship between EETs and prognosis of ASCVD has never been evaluated and remains unclear. The analysis did not demonstrate any statistically significant associations between eicosanoid metabolite concentrations at baseline and future MACE + revasc events. Secondary analyses revealed that participants in low/medium sum EETs and sum EETs + DHETs tertiles may exhibit a higher risk of experiencing a future MACE + revasc event. However, the trend observed with sum EETs was not statistically significant, and thus, these results should be interpreted with caution. If a relationship between EETs and occurrence of MACE + revasc events exists, these data suggest the observed association between lower plasma EET concentrations and higher risk of MACE + revasc events may be attributed to lower CYP epoxygenase function rather than higher sEH function.

It is well-established that CYP-derived EETs exhibit potent anti-inflammatory effects through lessening of endothelial activation and leukocyte adhesion via nuclear factor kappa B (NF-κB) inhibition[6,7], and exhibit potent vasodilatory properties through activation of Ca^{2+}-sensitive K^+ channels (BK_{Ca}) resulting in hyperpolarization[8]. EETs also stimulate endothelial cell growth and survival via activation of phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling[9], as well as reduce ischemia/reperfusion injury of the myocardium by a variety of mechanisms[10]. Increasing EET levels by inhibition of sEH has shown antihypertensive effects[11,12], renal protective effects[11,13], and cardiac protective effects[14] in multiple preclinical models of cardiovascular disease, including ischemia-reperfusion injury, by promoting the anti-inflammatory, vasodilatory and cellular protective properties of EETs. Although there is substantial preclinical evidence supporting the cardiovascular protective effects of EETs, these effects in human models of ASCVD remain poorly understood and require further study.

Prior analyses in the current study population revealed that low circulating EETs and lower CYP epoxygenase function (lower sum EETs + DHETs) were significantly associated with the presence and severity of obstructive ASCVD at baseline; however, in contrast, higher sEH function (lower 14,15-EET:DHET ratio) was not associated with severity of ASCVD[22]. The gene encoding sEH, *EPHX2*, has been shown to have polymorphisms, which can affect sEH activity[15]. Variation in *EPHX2* is associated with increased incidence of ASCVD[18,23]. Notably, the gain-of-function polymorphism Lys55Arg is related to higher sEH activity and increased incidence of ASCVD[18]. Variations in the CYP2C9, CYP2C8, and CYP2J2 enzymes that metabolize arachidonic acid into EETs have also been associated with varying EET

concentrations and cardiovascular disease[19-21]. This evidence suggests that genetic or metabolite biomarkers could identify a target population predisposed to low EET concentrations and higher ASCVD risk. The current analysis of future clinical outcomes in this study population suggest that lower EET concentrations and lower CYP epoxygenase function may also be associated with a worsened prognosis of ASCVD over time. These data suggest that if a precision medicine strategy is attempted to target patients with low EETs and significant ASCVD, it may be better to target CYP epoxygenase function instead of the sEH enzyme in order to increase EET levels. A novel sEH inhibitor is now currently in phase 2 clinical trials for COPD[16,17,26], but could be considered as a therapy for ASCVD patients. More research will be required to determine if an sEH inhibitor may be useful in humans with lower EET levels at baseline and significant ASCVD.

The current study investigated the relationship between EET levels and prognosis of human ASCVD for the first time by utilizing a very unique set of preexisting data. Our use of an existing cohort of eicosanoid metabolite data, the largest known ASCVD cohort with such data, is exceptionally resourceful given the difficulty in quantifying EET and DHET metabolites. However, there are multiple limitations in the current study that should be addressed. First. this study is observational, and thus it is not possible to determine if EETs alone cause MACE + revasc over time. In addition, potential confounding factors that cannot be changed could be present and interfere with the relationship between EETs and MACE + revasc. In order to lessen the impact of ASCVD status at baseline, which is known to be associated with lower EET concentrations and higher risk of MACE + revasc, we conducted analyses primarily within the strata of 98 participants with significant ASCVD at baseline. We also adjusted for additional clinical and demographic factors that are predictive of MACE + revasc events and/or EET concentrations in future analyses to increase confidence in the observed associations.

Another limitation of this study is the small sample size which may lead to false positives and unreliable results. Larger populations are required to validate results in the future. Lastly, in parallel to the metabolism of arachidonic acid to EETs by CYP2J and CYP2C, the CYP4F and CYP4A families metabolize arachidonic acid into 20-hydroxyeicosatraenoic acid (20-HETE)[27,28], which has vasoconstrictive and pro-inflammatory effects[4]. The EET concentrations may not be uniquely associated with MACE + revasc events over time as compared to other metabolites such as 20-HETE. This can be addressed with future analyses by looking at the available metabolite panel as a whole to determine relative associations between other metabolites and MACE + revasc.

A key novel feature of this study is its focus on precision medicine in the cardiovascular

field. While the precision medicine approach to therapy has already been successful in targeting novel therapies to distinct oncogenic mechanisms[2] for cancer treatment, it has not been as largely utilized in treatment of ASCVD. This research helps to elucidate information related to the three tested biomarkers (EET concentrations, CYP epoxygenase function, sEH activity) prompting new therapeutic approaches for ASCVD patients. The sum EETs + DHETs (CYP epoxygenase function) and 14,15-EET:DHET ratio (sEH function) were analyzed to obtain insight into an underlying mechanism behind low concentrations of EETs (sum EETs). CYP epoxygenase function, rather than sEH function, seem to be the more likely driver behind the association between lower EETs and higher risk of MACE + revasc events. If sEH function was found to be the driving force behind an association of low EETs and increased future MACE + revasc, then sEH inhibitors could be investigated for cardiovascular indications in addition to the indications for which they are already being tested. The sEH enzyme has already been utilized as a potential therapeutic target for inflammatory diseases[16,26], such as COPD, as evidenced by phase 2 clinical trials. If CYP epoxygenase activity is found to be the driving force, then this could be the focus of future studies as well as more viable therapeutic strategies such as structural analogs of EETs[29] rather than sEH inhibitors for ASCVD patients predisposed to low EET biosynthesis. The relationship between sEH or CYP epoxygenase function and various genotypes could be identified and used as indicators for those who may benefit from precision medicine for ASCVD.

Conclusions

Despite current therapies, ASCVD is still a major public health problem in the United States[1]. Though we can use genetic testing to diagnose and risk-stratify patients with cardiovascular disease, therapeutically targeting populations based on genotyping has not yet been utilized in the cardiovascular field[3]. Candidate pathways for the development of a precision medicine strategy in ASCVD are needed[3]. In this study, a unique cohort with preexisting eicosanoid data[22] was used to investigate the relationship between EET concentrations and ASCVD prognosis. Patients with existing ASCVD, lower EETs, and lower CYP epoxygenase function at baseline appeared to have a higher risk of future cardiovascular events. In contrast, sEH function was not associated with risk of events over time. Therefore, if a relationship between lower EETs and higher risk of future events exists, lower CYP epoxygenase function may be the driver instead of higher sEH function. However, these results should be interpreted with caution. Ultimately, larger populations will be needed to further investigate the relationship between EETs and ASCVD prognosis. The current study sets the stage for future research in this field, which may lead to future precision medicine strategies in ASCVD.

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Tables and Figures

Table 1: Description of the study population

Characteristic	Study Population	Strata with ASCVD at baseline
N	141	98
Age (years)	64 (55-70)	65 (53-71)
Female	64 (45.4%)	41 (41.8%)
African American race	30 (21.3%)	16 (16.3%)
BMI (kg/m^2)	29.8 (26.6-36.3)	29.9 (27.0-36.1)
Obese BMI ≥ 30.00	68 (48.2%)	48 (49.0%)
Current/recent (<1 year) smoker	38 (27.0%)	29 (29.6%)
Past medical history		
History of hypertension	112 (79.4%)	78 (79.6%)
History of diabetes	43 (30.5%)	35 (35.7%)
History of hyperlipidemia	94 (66.7%)	67 (68.4%)
Current heart failure	20 (14.2%)	14 (14.3%)
Previous myocardial infarction	22 (15.6%)	22 (22.5%)
Previous stroke or TIA	15 (10.6%)	12 (12.2%)
Previous Revascularization	48 (34.0%)	48 (49.0%)
Prior PCI	37 (26.2%)	37 (37.8%)
Prior CABG	15 (10.6%)	15 (15.3%)
Cardiac catheterization lab		
ACS on presentation	26 (18.4%)	25 (25.5%)
Presence of collateral	20 (14.2%)	20 (20.4%)
Current obstructive ASCVD[a]	68 (48.2%)	68 (69.4%)
Stenosis in most severe vessel (%)	60 (20-90)	80 (50-91)
Significant ASCVD[b]	98 (69.5%)	98 (100%)

BMI = body mass index, TIA = transient ischemic attack, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, ACS = acute coronary syndrome

Data presented as median (interquartile range) or count (proportion)

[a]Defined as a current ≥ 50% stenosis in the left main coronary artery or ≥ 70% stenosis in one or more of the non-left main coronary arteries during the index angiography procedure

[b]Defined as a current ≥ 50% stenosis or prior revascularization (PCI or bypass graft) in a major coronary artery

Table 2: Incidence of future MACE + revasc over a 4-year time period

Variables of Interest	Study Population	Strata with ASCVD at baseline
N	141	98
MACE + revasc	41 (29.1%)	37 (37.8%)
Death (all cause)	3 (2.1%)	2 (2.0%)
Non-fatal ACS	12 (8.5%)	11 (11.2%)
UA	2 (1.4%)	2 (2.0%)
NSTEMI	10 (7.1%)	9 (9.2%)
STEMI	0	0
Non-fatal stroke or TIA	4 (2.8%)	3 (3.1%)
Stroke	3 (2.1%)	2 (2.0%)
TIA	1 (0.7%)	1 (1.0%)
Revascularization procedure	21 (14.9%)	21 (21.4%)
PCI	18 (12.8%)	18 (18.4%)
CABG	3 (2.1%)	3 (3.1%)
Length of Follow-up (years)	3.8 (2.8-4.0)	3.8 (3.1-4.0)
Time to MACE + revasc or last follow-up (years)	3.2 (1.0-4.0)	2.5 (0.8-3.9)

MACE = major adverse cardiovascular events, revasc = revascularization procedures, ACS = acute coronary syndromes, UA = unstable angina, NSTEMI = non-ST elevated myocardial infarction, TIA = transient ischemic attack, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft

Data presented as median (interquartile range) or count (proportion).

Table 3: Analysis of clinical factors across sum EET tertiles in the ASCVD population

Characteristic	Low Sum EETs (N=33)	Medium Sum EETs (N=32)	High Sum EETs (N=33)	p-value
Age (years)	64 (51.5-68)	67.5 (57.3-73.8)	68 (59.5-71.5)	p=0.025
Age > 65 years old	12 (36.4%)	18 (56.3%)	18 (54.6%)	p=0.200
Female	14 (42.4%)	14 (43.8%)	13 (39.4%)	p=0.935
African American race	3 (9.1%)	8 (25.0%)	5 (15.2%)	p=0.216
BMI (kg/m^2)	34.7 (28.8-37.0)	28.6 (26.0-34.2)	29.2 (25.7-36.5)	p=0.088
Obese BMI ≥ 30.00	22 (66.7%)	12 (37.5%)	14 (42.4%)	p=0.039
Current/recent (<1 year) smoker	8 (24.2%)	14 (43.8%)	7 (21.2%)	p=0.104
Past medical history				
History of hypertension	27 (81.8%)	27 (84.4%)	24 (72.7%)	p=0.542
History of diabetes	12 (36.4%)	13 (40.6%)	10 (30.3%)	p=0.681
History of hyperlipidemia	24 (72.7%)	19 (59.4%)	24 (72.7%)	p=0.419
Current heart failure	6 (18.2%)	5 (15.6%)	3 (9.1%)	p=0.592
Previous myocardial infarction	9 (27.3%)	7 (21.9%)	6 (18.2%)	p=0.674
Previous stroke or TIA	6 (18.2%)	4 (12.5%)	2 (6.1%)	p=0.323
Previous Revascularization	21 (63.6%)	12 (37.5%)	15 (45.5%)	p=0.094
Prior PCI	18 (54.6%)	9 (28.1%)	10 (30.3%)	p=0.051
Prior CABG	6 (18.2%)	3 (9.4%)	6 (18.2%)	p=0.569
Cardiac catheterization lab				
ACS on presentation	10 (30.3%)	3 (9.4%)	12 (36.4%)	p=0.028
Presence of collateral	10 (30.3%)	6 (18.8%)	4 (12.1%)	p=0.193
Current obstructive ASCVD[a]	25 (75.8%)	21 (65.6%)	22 (66.7%)	p=0.612
Stenosis in most severe vessel (%)	90 (60-92.5)	75 (50-90)	80 (55-95)	p=0.287

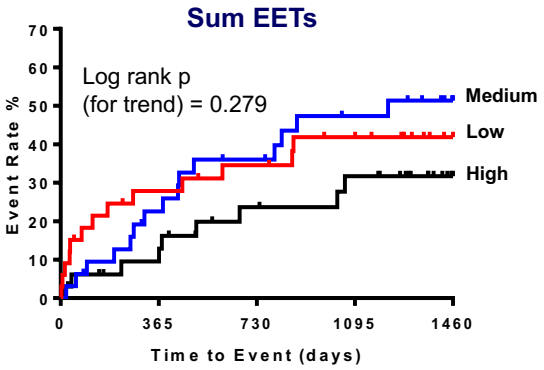
BMI = body mass index, TIA = transient ischemic attack, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, ACS = acute coronary syndrome

Data presented as median (interquartile range) or count (proportion)

[a]Defined as a current ≥ 50% stenosis in the left main coronary artery or ≥ 70% stenosis in one or more of the non-left main coronary arteries

Table 4: Comparison of Eicosanoid Metabolites at baseline across future MACE + Revasc status in the ASCVD population

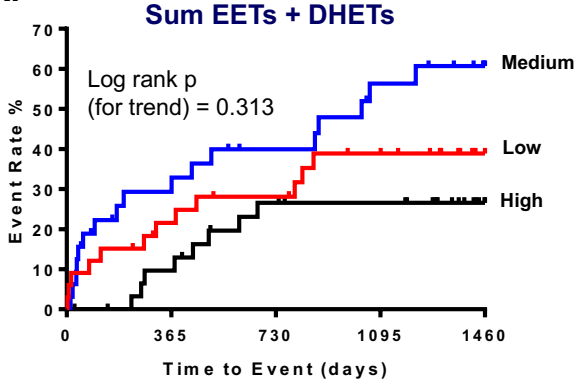
Eicosanoid Biomarkers	MACE + revasc (yes) (N=37)	MACE + revasc (no) (N=61)	p-value
Sum EETs (ng/mL)	0.61 (0.52-0.71)	0.66 (0.54-0.83)	0.406
Sum EETs + DHETs (ng/mL)	2.63 (2.19-3.05)	2.75 (2.12-3.42)	0.363
14,15-EET:DHET ratio (ng/mL)	0.30 (0.23-0.42)	0.34 (0.25-0.42)	0.641



	# Events / N (%)	Adjusted HR (95% CI)	P-value
High	9 / 33 (27 %)	Reference	--
Medium	15 / 32 (47 %)	2.03 (0.85-5.10)	0.110
Low	13 / 33 (39 %)	1.32 (0.53-3.39)	0.553
Low/Med	28 / 65 (43 %)	1.64 (0.78-3.80)	0.201

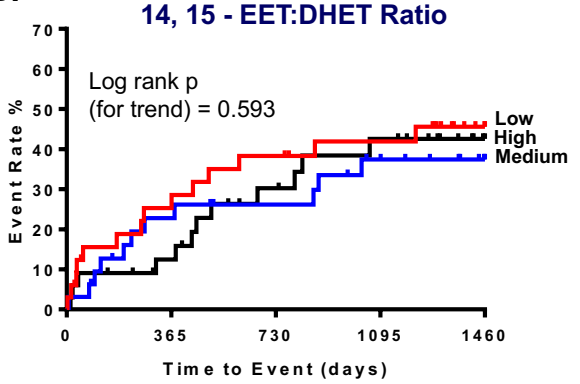
Figure 1. **Sum EET levels at baseline and risk of future MACE + revasc in the ASCVD population.** Kaplan Meier curves were created for incidence of MACE + revasc for sum EET tertiles. The unadjusted log rank p-value for trend across tertiles is shown. Next to the curve, the number of events and event rates within each tertile are provided. Adjusted hazards ratios (HR), 95% confidence intervals (CI), and p-values are shown next to the event rates. The event number/rates, HR, 95% CI, and p-values are also reported for the low and medium tertiles combined (low/med) to compare against the high tertile.

A.



	# Events /N (%)	Adjusted HR (95% CI)	P-value
High	8 / 33 (24 %)	Reference	--
Medium	17 / 32 (53 %)	2.58 (1.05-6.82)	0.038
Low	12 / 33 (36 %)	1.51 (0.58-4.08)	0.400
Low/Med	29 / 65 (45 %)	1.98 (0.90-4.87)	0.094

B.



	# Events / N (%)	Adjusted HR (95% CI)	P-value
High	12 / 33 (36 %)	Reference	--
Medium	11 / 32 (34 %)	1.03 (0.42-2.50)	0.950
Low	14 / 33 (42 %)	1.09 (0.49-2.45)	0.834
Low/Med	25 / 65 (38 %)	1.06 (0.52-2.27)	0.866

Figure 2. **Eicosanoid metabolite levels at baseline and risk of future MACE + revasc in the ASCVD population.** Kaplan Meier curves were created for incidence of MACE + revasc corresponding to tertiles for baseline sum EETs + DHETs (A) and 14,15-EET:DHET ratios (B). On each curve, the unadjusted log rank p-value for trend across tertiles is shown. Next to the curves, the number of events and event rates within each tertile are provided. Adjusted hazards ratios (HR), 95% confidence intervals (CI), and p-values are shown next to the event rates. The event number/rates, HR, 95% CI, and p-values are also reported for the low and medium tertiles combined (low/med) to compare against the high tertiles.

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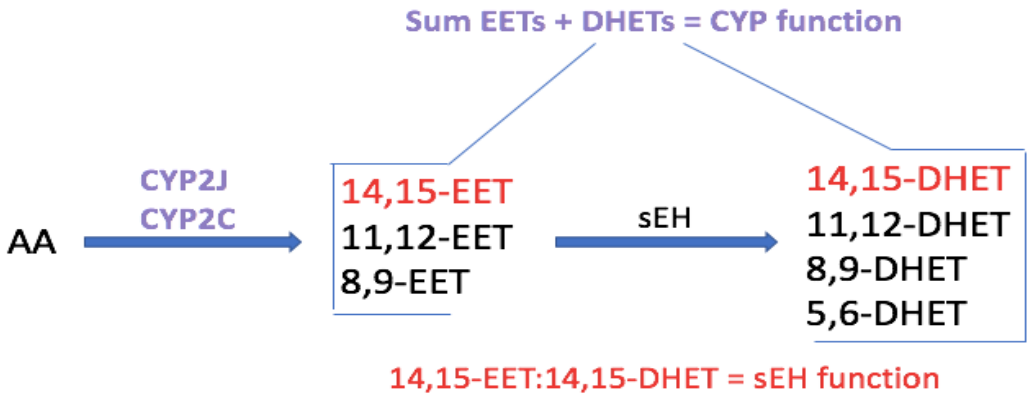
Conflicts of Interest

The authors have no conflicts of interest to disclose.

Supplemental Materials

Supplemental Figure 1: Biosynthesis and metabolism of epoxyeicosatrienoic acids (EETs). Cytochrome-P450 (CYP) enzymes metabolize arachidonic acid (AA) into bioactive EET regioisomers. EETs are then further metabolized into less active dihydroxyeicosatrienoic acid (DHETs) regioisomers by soluble epoxide hydrolase (sEH). CYP function is represented by sum EETs + DHETs because AA is metabolized by CYPs into EETs and then further metabolized into DHETs, and so the sum of EETs + DHETs is a biomarker of downstream effects of CYPs. The 14,15-EET:DHET ratio is representative of sEH function because 14,15-EETs are the preferred substrate of sEH and 14,15-EET is metabolized by sEH into 14,15-DHET.



Supplemental Table 1: Comparison of clinical factors across MACE + revasc event status in patients with significant ASCVD

Characteristic	MACE + revasc (yes) (N=37)	MACE + revasc (no) (N=61)	p-value	HR (95% CI)	p-value
Age > 65 years old	17 (48.7%)	30 (49.2%)	0.959	1.05 (0.55-2.00)	p=0.888
Female	17 (46.0%)	24 (39.3%)	0.521	1.12 (0.58-2.14)	p=0.521
African American race	6 (16.2%)	10 (16.4%)	0.982	1.04 (0.39-2.32)	p=0.982
Obese BMI ≥ 30.00	19 (51.4%)	29 (47.5%)	0.715	1.09 (0.57-2.10)	p=0.715
Current/recent (<1 year) smoker	14 (37.8%)	15 (24.6%)	0.167	1.33 (0.67-2.56)	p=0.167
Past medical history					
History of hypertension	32 (86.5%)	46 (75.4%)	0.177	1.89 (0.81-5.54)	p=0.177
History of diabetes	16 (43.2%)	19 (31.2%)	0.228	1.54 (0.79-2.95)	p=0.228
History of hyperlipidemia	26 (70.3%)	41 (67.2%)	0.752	1.16 (0.59-2.44)	p=0.752
Current heart failure	7 (18.9%)	7 (11.5%)	0.314	1.32 (0.53-2.84)	p=0.314
Previous myocardial infarction	10 (27.0%)	12 (19.7%)	0.401	1.31 (0.60-2.61)	p=0.401
Previous stroke or TIA	4 (10.8%)	8 (13.1%)	0.734	0.80 (0.24-2.01)	p=0.734
Previous Revascularization	24 (64.9%)	24 (39.3%)	0.014	2.08 (1.08-4.22)	p=0.014
Prior PCI	19 (51.4%)	18 (29.5%)	0.031	2.06 (1.08-3.97)	p=0.031
Prior CABG	7 (18.9%)	8 (13.1%)	0.444	1.11 (0.45-2.38)	p=0.444
Cardiac catheterization lab					
ACS on presentation	10 (27.0%)	15 (24.6%)	0.789	1.09 (0.51-2.20)	p=0.789
Presence of collateral	7 (18.9%)	13 (21.3%)	0.775	0.94 (0.38-2.00)	p=0.775
Current obstructive ASCVD[a]	28 (75.7%)	40 (65.6%)	0.288	1.66 (0.81-3.73)	p=0.566
Stenosis in most severe vessel (%)	90 (60-92.5)	80 (50-92.5)	0.224	----------	--------

BMI = body mass index, TIA = transient ischemic attack, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, ACS = acute coronary syndrome

Data presented as median (interquartile range) or count (proportion)

[a]Current obstructive coronary artery disease (CAD) defined as ≥ 50% stenosis in the left main coronary artery or ≥ 70% stenosis in one or more of the non-left main coronary arteries

Supplemental Table 2: Analysis of clinical factors across sum EETs + DHETs tertiles in the ASCVD population

Characteristic	Low Sum EETs + DHETs (N=33)	Medium Sum EETs + DHETs (N=32)	High Sum EETs + DHETs (N=33)	p-value
Age (years)	65 (52-69)	66 (60-72.8)	65 (54-70.5)	p=0.138
Age >65 years old	15 (45.5%)	17 (53.1%)	16 (48.5%)	p=0.824
Female	14 (42.4%)	18 (56.3%)	9 (27.3%)	p=0.058
African American race	5 (15.2%)	8 (25.0%)	3 (9.1%)	p=0.216
BMI (kg/m^2)	34.4 (28.9-36.6)	29.5 (27.0-37.2)	27.8 (25.1-34.0)	p=0.060
Obese BMI ≥ 30.00	21 (63.6%)	14 (43.8%)	13 (39.4%)	p=0.109
Current/recent (<1 year) smoker	5 (15.2%)	13 (40.6%)	11 (33.3%)	p=0.057
Past medical history				
History of hypertension	26 (78.8%)	29 (90.6%)	23 (36.7%)	p=0.115
History of diabetes	11 (33.3%)	14 (43.8%)	10 (30.3%)	p=0.499
History of hyperlipidemia	21 (63.6%)	24 (75.0%)	22 (66.7%)	p=0.590
Current heart failure	7 (21.2%)	3 (9.4%)	4 (12.1%)	p=0.445
Previous myocardial infarction	8 (24.2%)	6 (18.8%)	8 (24.2%)	p=0.826
Previous stroke or TIA	5 (15.2%)	3 (9.4%)	4 (12.1%)	p=0.926
Previous Revascularization	18 (54.6%)	15 (46.9%)	15 (45.5%)	p=0.730
Prior PCI	15 (45.5%)	12 (37.5%)	10 (30.3%)	p=0.445
Prior CABG	5 (15.2%)	4 (12.5%)	6 (18.2%)	p=0.938
Cardiac catheterization lab				
ACS on presentation	9 (27.3%)	7 (21.9%)	9 (27.3%)	p=0.845
Presence of collateral	8 (24.2%)	5 (15.6%)	7 (21.2%)	p=0.677
Current obstructive ASCVD[a]	24 (72.7%)	25 (78.1%)	19 (57.6%)	p=0.178
Stenosis in most severe vessel (%)	90 (60-90)	80 (52.5-93.8)	70 (50-95)	p=0.311

BMI = body mass index, TIA = transient ischemic attack, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, ACS = acute coronary syndrome

Data presented as median (interquartile range) or count (proportion)

[a]Current obstructive coronary artery disease (CAD) defined as ≥ 50% stenosis in the left main coronary artery or ≥ 70% stenosis in one or more of the non-left main coronary arteries

Supplemental Table 3: Analysis of clinical factors across 14,15-EET:DHET ratio tertiles in the ASCVD population

Characteristic	Low 14,15-EET:DHET Ratio (N=33)	Medium 14,15-EET:DHET Ratio (N=32)	High 14,15-EET:DHET Ratio (N=33)	p-value
Age (years)	65 (53-68.5)	62.5 (51.3-69.8)	68 (60-75)	p=0.030
Age > 65 years old	16 (48.5%)	13 (40.6%)	19 (57.6%)	p=0.390
Female	13 (39.4%)	16 (50.0%)	12 (36.4%)	p=0.507
African American race	4 (12.1%)	5 (15.6%)	7 (21.2%)	p=0.642
BMI (kg/m^2)	31.7 (25.8-37.2)	30.1 (27.8-37.5)	28.9 (26.4-35.2)	p=0.407
Obese BMI ≥ 30.00	18 (54.6%)	16 (50.0%)	14 (42.4%)	p=0.609
Current/recent (<1 year) smoker	13 (39.4%)	8 (25.0%)	8 (24.2%)	p=0.325
Past medical history				
History of hypertension	28 (84.9%)	23 (71.9%)	27 (81.8%)	p=0.409
History of diabetes	13 (39.4%)	12 (37.5%)	10 (30.3%)	p=0.716
History of hyperlipidemia	27 (81.8%)	20 (62.5%)	20 (60.6%)	p=0.109
Current heart failure	5 (15.2%)	5 (15.6%)	4 (12.1%)	p=0.938
Previous myocardial infarction	9 (27.3%)	6 (18.8%)	7 (21.2%)	p=0.700
Previous stroke or TIA	6 (18.2%)	4 (12.5%)	2 (6.1%)	p=0.323
Previous Revascularization	20 (60.6%)	11 (34.4%)	17 (51.5%)	p=0.097
Prior PCI	16 (48.5%)	9 (28.1%)	12 (36.4%)	p=0.233
Prior CABG	6 (18.2%)	2 (6.3%)	7 (21.2%)	p=0.205
Cardiac catheterization lab				
ACS on presentation	7 (21.2%)	10 (31.3%)	8 (24.2%)	p=0.640
Presence of collateral	9 (27.3%)	8 (25.0%)	3 (9.1%)	p=0.140
Current obstructive ASCVD[a]	23 (69.7%)	22 (68.8%)	23 (69.7%)	p=0.996
Stenosis in most severe vessel (%)	80 (55-95)	72.5 (50-90)	80 (60-90)	p=0.694

BMI = body mass index, TIA = transient ischemic attack, PCI = percutaneous coronary intervention, CABG = coronary artery bypass graft, ACS = acute coronary syndrome

Data presented as median (interquartile range) or count (proportion)

[a]Current obstructive coronary artery disease (CAD) defined as ≥ 50% stenosis in the left main coronary artery or ≥ 70% stenosis in one or more of the non-left main coronary arteries